The **hobo** family of transposable elements, one of three transposable-element families that cause hybrid dysgenesis in *Drosophila melanogaster*, appears to be present in all members of the *D. melanogaster* species complex: *D. melanogaster*, *D. simulans*, *D. mauritiana*, and *D. sechellia*. Some **hobo**-hybridizing sequences are also found in the other members of the *melanogaster* subgroup and in many members of the related *montium* subgroup. Surveys of older isofemale lines of *D. melanogaster* suggest that complete **hobo** elements were absent prior to 50 years ago and that **hobo** has recently been introduced into the species by horizontal transfer. To test the horizontal transfer hypothesis, the 2.6-kb *Xhol* fragments of **hobo** elements from *D. melanogaster*, *D. simulans*, and *D. mauritiana* were cloned and sequenced. The DNA sequences reveal an extremely low level of divergence and support the conclusion that the active **hobo** element has been horizontally transferred into or among these species in the recent past.

**Introduction**

The **hobo** elements are one of three transposable-element (TE) families (the others being the *P* and *I* families) known to cause hybrid dysgenesis in *Drosophila melanogaster* (for review, see Blackman and Gelbart 1989; Engels 1989; Finnegan 1989b). Structurally they fall into the class II, type I category of eukaryotic TEs, having short inverted repeats and probably transposing via a DNA intermediate (Finnegan 1989a). **Hobo** is thus structurally similar to the *P* element of *D. melanogaster*, the *Ac* element of *Zea mays*, and the *Tam3* element of *Antirrhinum majus*, and **hobo** appears to have some protein sequence similarity with *Ac* and *Tam3*, although not with *P* (Streck et al. 1986; Calvi et al. 1991). A functional **hobo** element, *Hfll*, is 2,959 bp in length, has short inverted terminal repeats of 12 bp, and appears to code for a single polypeptide (Calvi et al. 1991). **Hobo** elements are heterogeneous in size, and a typical genome contains 2–10 copies of the full-length sequence and as many as 50 copies of smaller elements that appear to be derived from the complete sequence by internal deletion. Insertion sites of **hobo** elements are typically flanked by an 8-bp duplication of host sequence (Mcginnis et al. 1983; Streck et al. 1986).

Hybrid dysgenesis caused by **hobo** elements bears a strong resemblance to that caused by *P* elements. When male flies of certain lines whose genomes contain complete **hobo** elements are mated to females whose genomes lack them, the typical stigmata of hybrid dysgenesis—gonadal dysgenesis and high rates of mutation—are observed.
in the germ line of the F₁ (Blackman et al. 1987; Yannopoulos et al. 1987). However, both the reciprocal cross-effect and the overall rates of mutation and other chromosomal damage are less extreme in hobo than in P-M hybrid dysgenesis (Blackman and Gельbart 1989).

The evolutionary histories of hobo and P appear to be different, despite their obvious structural and functional similarities. Within the family Drosophilidae, P sequences are found in the melanogaster, willistoni, saltans, obscura, and immigrans species groups of the genus Drosophila, as well as in the genus Scaptomyza, but are found in only a single species of the melanogaster subgroup, D. melanogaster itself (Lansman et al. 1985; Stacey et al. 1986; Daniels et al. 1990b). In contrast, hobo sequences are restricted to the melanogaster species group but are widely distributed among species of two of its subgroups, montium and melanogaster (Daniels et al. 1990a).

The distribution of hobo elements in natural populations has been studied chiefly through restriction surveys of genomic DNAs extracted from isofemale lines, which detect the presence of the 2.6-kb XhoI fragment characteristic of supposedly complete hobo elements. Streck et al. (1986) examined a few strains of D. melanogaster, D. simulans, and D. mauritiana and found evidence of this fragment in all samples from the latter two species. However, of the six strains of D. melanogaster they examined, only three (Oregon R-B, Amherst 3, and Daek) contained the 2.6-kb fragment and were dubbed "H" (for hobo) strains. Three others (Samarkand, Gaiano, and Tunnelgaten) lacked it. These three "empty" (E) strains did, however, contain bands >8 kb which hybridized to a hobo probe.

More extensive surveys of hobo elements in natural populations show that hobo elements are widespread in D. melanogaster and D. simulans (Periquet et al. 1989a, 1989b, and accepted; Boussy and Daniels 1991; Pascual and Periquet 1991). Both species contain both H and E strains, and both H and E strains contain a wide variety of hobo-hybridizing bands that are >2.6 kb in an Xhol digest. In all cases in D. melanogaster the E strains are from older collections, while all recently collected strains are H strains. In D. simulans there are virtually no strains whose exact collection dates are known, so it is not possible to determine whether the E strains observed in this species are also the older ones (Boussy and Daniels 1991). Very few strains of D. mauritiana have been examined, but all contain the 2.6-kb fragment, as well as smaller and larger bands. One strain of D. sechellia has also been examined by two different groups of investigators. Daniels et al. (1990a) failed to find the 2.6-kb Xhol band in this species, but Periquet et al. (accepted) did observe it.

On the basis of the correlation between collection date and presence of the 2.6-kb Xhol fragment in strains of D. melanogaster, Pascual and Periquet (1991) have proposed that the "complete" hobo element has a history of recent horizontal introduction similar to that of the P element, and they suggested D. simulans as the possible source for the sequence. Boussy and Daniels (1991) agreed with the horizontal transfer hypothesis, but the presence of E strains in their samples of D. simulans raised doubts about the direction of transfer.

One way to test the horizontal transfer hypothesis for any TE family is to examine DNA sequences of functional elements from the supposed donor and recipient species. If the phylogenetic relationship among the species is known, one can predict, on the basis of molecular analyses of nuclear genes, approximately how much sequence divergence will have accumulated if the elements (a) were present in the common ancestor and (b) diverged within each lineage independently. Levels of divergence of
TE sequences which are substantially lower than those for ordinary nuclear genes suggest a more recent common ancestor for the TE sequences than for the lineages themselves and may be evidence for a horizontal transfer event. Such evidence has been used to support the recent horizontal introduction of the P element into D. melanogaster from the willistoni group (Daniels et al. 1990b).

I have sequenced the 2.6-kb Xhol fragment of a complete hobo element from each of three species in the D. melanogaster species complex: D. melanogaster, D. simulans, and D. mauritiana. The phylogenetic relationships among these species are reasonably well understood on the basis of biogeographic (Lachaise et al. 1988), cytological (Lemeunier et al. 1986), and molecular data (Cariou 1987; Caccone et al. 1988). A comparison between the phylogeny of the complex and the DNA sequences of hobo elements cloned from these three species should therefore be informative about the history of hobo elements in these species.

Material and Methods
Fly Stocks and Culture Conditions

Fly stocks used to extract DNA for genomic library construction were established from single wild-caught females, whose progeny were kept in mass bottle culture on standard cornmeal medium at 18-25°C. The Drosophila melanogaster stock OK49 was from a collection called “Okavanga” made by Rob Dorit in 1985 in Botswana. The D. simulans stock RA5 came from a collection that I made at the Raleigh, N.C. Farmers’ Market in 1986. The stock of D. mauritiana was provided by Jerry Coyne.

Preparation of Genomic DNA

Adult flies from the stocks were collected shortly after the lines were established in the laboratory and were frozen at −70°C until processed for DNA extraction. Approximately 1 g of flies from each stock was used for each extraction. High-molecular-weight genomic DNA was prepared using a cesium chloride gradient method (Bingham et al. 1982). The presence of putative full-length hobo elements in each line was confirmed by hybridizing the plasmid pH108, which contains a full-length hobo element whose DNA sequence is known (Mcginnis et al. 1983; Streck et al. 1986), to Southern blots of Xhol-digested genomic DNAs, by using standard methods (Sambrook et al. 1989, pp. 9.42–9.55). The presence of a strong 2.6-kb band was taken as evidence of the presence of full-length hobo elements in the stocks used for library construction (data not shown).

Library Construction, Screening, and Subcloning

Genomic libraries for each stock were made in the bacteriophage lambda vector EMBL4 by using genomic DNAs digested with BamHI, which does not cut within the canonical hobo element (Mcginnis et al. 1983). The library screen was performed using standard plaque lift techniques (Sambrook et al. 1989, pp. 2.108–2.117). A two-tiered approach was used. Each library was initially screened with the purified 2.6-kb Xhol fragment of pH108, a plasmid containing a complete hobo element. Once positive phages had been identified with this probe, they were further probed with the 900-bp EcoRI fragment of the same plasmid. Since most of the incomplete, internally deleted hobo elements identified by Streck et al. (1986) lack the EcoRI fragment completely, this second screen helped to ensure that those phages selected for subcloning contained full-length, potentially functional hobo elements. Phages that were positive after the second screen were characterized by restriction digestion with XhoI and EcoRI,
followed by Southern blotting and hybridization to a probe made from the pH108 plasmid, to assay for the presence of hobo-homologous bands. The presence of both a 2.6-kb band in the XhoI digest and a 900-bp band in the EcoRI digest was taken as evidence that the positive phage contained a full-length hobo element. The 2.6-kb XhoI band from each positive phage was subcloned into the phagemid pBluescript (Stratagene) for DNA sequencing.

Probe DNAs

Probe DNAs were prepared by digesting the plasmid pH108 with the appropriate restriction endonuclease and then purifying the desired fragment by electroelution using an Elutrap (Schleicher and Schuell). Probes were labeled by nick-translation using $\alpha^{32}$P dCTP as the label (Sambrook et al. 1989, pp. 10.6–10.10).

DNA Sequencing Reactions

The subcloned $Xh0l$ fragments of each cloned hobo element were sequenced directly from the double-stranded plasmids by using the Taqtrack DNA sequencing system (Promega). A series of 20-mer oligonucleotide primers for sequencing (North Carolina State University Molecular Biology Center or Research Genetics, Inc.) were constructed on the basis of the published sequence of a hobo element (hobo108; Streck et al. 1986) and were spaced approximately every 200 bases along both strands of the hobo element sequence. These primers were end-labeled with $\gamma^{32}$P ATP (3,000 Ci/mmoll; Dupont NEN), according to the protocol provided in the Taqtrack kit. Sequencing reactions were carried out according to kit instructions, with modifications as follows: ~4 $\mu$g of plasmid DNA was mixed with 2 $\mu$l of end-labeled primer mix in 1× $Taq$ polymerase buffer at a final volume of 25 $\mu$l. The template-primer mix was denatured at 95°C on a heating block for 5 min and then was allowed to anneal at 37°C in a water bath for 10 min. After the annealing, 1.6 $\mu$l (4 units) of sequencing-grade $Taq$ polymerase was added to the annealed mixture. Six microliters of this mix was dispensed to the side of each of four wells of a Stratatray (Stratagene), each of which contained 1 $\mu$l of the appropriate d/ddNTP mix. The Stratatray was centrifuged briefly in a salad spinner (Copco) equipped with a special carrier to start the reactions. The Stratatray was floated in a 70°C water bath for 10 min, after which 4 $\mu$l of stop mix was dispensed to the side of each well, and the tray was again centrifuged briefly. Completed reactions were stored at −20°C. Reactions were heated to 95°C for 5 min and then were placed on ice prior to being loaded onto the sequencing gel for electrophoresis. A total of 2–3 $\mu$l of each reaction mix was loaded into a lane.

Gel Electrophoresis, Drying, and Exposure

Sequencing gels were cast and run by standard methods using an electrolytic gradient (Sheen and Seed 1988). After electrophoresis, gels were dried for 1 h on a Bio-Rad gel drier and then were exposed to Kodak SB autoradiography film. Exposure times were 12 h–7 d, depending on the age of the end-labeling reaction mix.

Sequencing Strategy

Because sequencing errors have the potential to seriously inflate estimates of sequence divergence, especially when these levels are low, a multiply-redundant strategy was used which allowed each segment of each hobo element to be sequenced at least two times and as many as five times. Regions that differed from the published sequence of hobo108 were sequenced at least four times. As a further control for sequencing
artifacts, the complete hobo element hobo108 in the plasmid pH108, which was previously sequenced by Streck et al. (1986), was completely resequenced, and the results were compared with the published sequence.

Sequence Analysis

DNA sequence data were entered into a data base in the GCG DNA sequence analysis package (Devereaux et al. 1984) running on a Microvax computer, either by means of a digitizer (Graf/bar) or manually. Overlapping fragments were assembled using GELASSEMBLE and related routines. Sequence comparisons were performed with the BESTFIT routine, and sequences were aligned with the aid of the LINEUP routine.

![Diagram showing DNA sequences of hobo elements from various species](image)

**Fig. 1.**—DNA sequences of the 2.6-kb XhoI fragments of five hobo elements from the Drosophila melanogaster species complex. The top cartoon shows the structure of Hf11, a complete, functional hobo element described by Calvi et al. (1991). Features include the two XhoI sites which produce the 2.6-kb band diagnostic of complete hobo elements, the open reading frame (ORF1), the S repeat region, and the inverted repeats at each end of the element. The scale at the bottom shows the locations of features relative to the Hf11 sequence. OK, RA, and D. mauritiana are the sequences of 2.6-kb XhoI fragments of complete hobo elements from libraries of D. melanogaster, D. simulans, and D. mauritiana, respectively. Hoho108 is the sequence (as determined by G.M.S.) of a complete but possibly nonfunctional hobo element cloned by McGinnis et al. (1983) and previously sequenced by Streck et al. (1986). Nucleotide sites are numbered according to the method of Calvi et al. (1991). Capital letters in each sequence show the character states at nucleotide positions which differed between any two sequences, and indel variation is shown relative to the Hf11 sequence.
Results

Figure 1 presents the sequences of the XhoI fragments of three hobo elements—from Drosophila melanogaster, D. simulans, and D. mauritiana—alongside the sequences of hobo108 (as resequenced here) and Hf11 as described by Calvi et al. (1991). Only five sites in the coding region, all within a 500-bp region of the left-hand part of ORF1, are different from those of Hf11. Two of these, position 573 in hobo108 (D. melanogaster) and position 635 in RA (D. simulans), are silent substitutions. The other three all lead to an amino acid replacement: in OK (D. melanogaster) at position 659, an A → C mutation replaces an asparagine residue by a threonine residue. In the D. mauritiana subclone, a T → C shift at position 610 replaces cysteine by arginine, while at position 1052 an A → G shift replaces tyrosine by cysteine. There are two nucleotide differences from Hf11 in the 3’ noncoding region, both in the RA (D. simulans) subclone. There are no frameshift or nonsense mutations.

Only the hobo108 element shows any indel variation from Hf11. As described by Calvi et al. (1991), there is an insertion of seven perfect copies of the 9-bp S repeat sequence in hobo108 at position 1903 within the coding region. This has the effect of adding 21 amino acids—seven repeats of the sequence threonine-proline-glutamic acid—to the deduced protein product of ORF1. At position 2709 there is a 5-bp insert relative to Hf11 (GACCA), but this insertion falls into a portion of the noncoding region shown to be unnecessary for hobo transposition. Germ-line transformation experiments using the hobo108 element have not conclusively shown this element to be functional (Calvi et al. 1991).

Two of the differences that Calvi et al. (1991) noted between Hf11 and hobo108 are apparently due to errors in the published sequence of hobo108 (Streck et al. 1986). These are the substitution of CC for TT at positions 1978-1979 (the correct sequence here in all hobo elements is CC) and the insertion of a C at position 2221, which generates a PvuII restriction site which is present in all sequences (Calvi et al. 1991).

Discussion

There are two approaches to identifying the possibility of horizontal transfer of TE sequences among taxa: one approach is to look for discontinuities in the phylogenetic or intraspecific distribution of the element; and the other approach is to sequence copies of the element from related species and compare the between-elements divergence levels with those typical for ordinary genes in those species. Daniels et al. (1990a) surveyed the genus Drosophila to examine the phylogenetic pattern of occurrence of hobo sequences. Within the melanogaster subgroup, which contains D. melanogaster and seven sibling species, they confirmed the presence of many hobo-hybridizing sequences within D. melanogaster, D. simulans, D. mauritiana, and D. sechellia. They also observed hybridization to genomic DNA from three other members of the subgroup—D. teissieri, D. yakuba, and D. erecta (the eighth species of the subgroup, D. orena, was not examined)—but none of these species presented the characteristic 2.6-kb XhoI fragment of a complete hobo element, and the hybridization was weak.

In the genus Drosophila, the only other group that appears to contain hobo sequences is the montium subgroup. The melanogaster and montium subgroups are both part of the melanogaster species group, but the two subgroups are probably not sister taxa (Lemeunier et al. 1986). The montium subgroup comprises 79 species, 26 of
which were surveyed by Daniels et al. (1990a). Of these, 23 hybridized weakly with a *D. melanogaster* *hobo* probe, but none displayed the 2.6-kb *XhoI* fragment.

A simple model of *hobo* evolution in the genus *Drosophila* can be built upon the presence or absence of *hobo*-like sequences in the species examined by Daniels et al. (1990a). In such a model, *hobo* entered the common ancestor of the *melanogaster* and *montium* subgroups and has been vertically (phylogenetically) transmitted within most species of these two lineages while going extinct in all others. The presence of the 2.6-kb *XhoI* band only in members of the *D. melanogaster* species complex is consistent with this picture. Its absence from the other members of the *melanogaster* subgroup may reflect either sequence divergence of functional elements or loss of the functional element in the branch leading to *D. teissieri*, *D. yakuba*, and *D. erecta*. Likewise, the absence of the 2.6-kb band from *montium*-subgroup flies is unsurprising, given that the *melanogaster* and *montium* subgroups have been separated since early in the Sophophoran radiation, ~35 Mya (Throckmorton 1975; Lemeunier et al. 1986).

The DNA sequences of *hobo* elements from the *D. melanogaster* complex are at odds with this simple model. The paucity of nucleotide differences between *hobo* elements derived from *D. melanogaster*, *D. simulans*, and *D. mauritiana*, as shown in table 1, is quite surprising if active *hobo* elements were once present in the common ancestor of the species complex and have been evolving independently in each member of the complex. The cosmopolitan *D. melanogaster* and *D. simulans* are probably separated by a minimum of 0.8–3 Myr of evolution, while the island-endemic *D. mauritiana* was derived from *D. simulans* ~0.4–1 Mya (Cariou 1987; Caccone et al. 1988; Lachaise et al. 1988). Ordinary genetic loci such as *Adh*, *Mtn*, and the *y-ac-sc* region, whose sequences have been compared both within and between *D. melanogaster* and *D. simulans*, typically differ at ~0.1%–0.6% of their silent nucleotide sites, within species (Kreitman and Aguadé 1986; Aguadé et al. 1989; Lange et al. 1990; Martín-Campos et al. 1992). In contrast, these loci differ at ~5% of their silent nucleotide positions, between *D. melanogaster* and *D. simulans* (Bodmer and Ashburner 1984; Cohn and Moore 1988; Lange et al. 1990; Martín-Campos et al. 1992). The *hobo* sequences from different species differ by 0.04%–0.2% of all nucleotide sites (table 1) and thus appear to be similar in age to alleles within a species. Clearly, the divergence

Table 1

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<tr>
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<th><em>D. melanogaster</em></th>
<th><em>D. simulans</em></th>
<th><em>D. mauritiana</em></th>
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<td></td>
<td>HFII</td>
<td>OK</td>
<td>hobo108</td>
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<td><em>D. melanogaster</em>:</td>
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<td>hobo108</td>
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<td><em>D. simulans</em>:</td>
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<td>RA</td>
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<td><em>D. mauritana</em>:</td>
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<td>maur</td>
<td>2</td>
<td>3</td>
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</table>

*NOTE.—Values above the diagonal are the proportional amount of difference between two sequences, and values below the diagonal are the absolute number of nucleotide differences between two sequences.*

*Entries of the form “a+b” indicate that there were a nucleotide differences and b insertions or deletions.*
of hobo elements in the complex is anomalous among nuclear genic sequences and is thus inconsistent with the hypothesis of vertical transmission.

One possible interpretation of the data is that the complete hobo sequence has, in fact, been vertically transmitted but that the sequence is highly constrained and can tolerate few substitutions, even at presumed silent sites. Information on the molecular biology of hobo elements and the structurally similar P elements militates against this idea. The work of Blackman et al. (1987) and Calvi et al. (1991) shows that hobo elements which either lack most of the 3' nontranscribed sequence or contain large insertions of exogenous sequence (marker genes) in this region can still function in germ-line transformation experiments. Yet, among the five hobo sequences shown in figure 1, there are only three differences in the 3' noncoding region: two base substitutions in the D. simulans sequence and one 5-bp deletion in hobo108.

A similar lack of sequence variation has been observed among P elements within D. melanogaster (O'Hare and Rubin 1983; Sakoyama et al. 1985; Black et al. 1987). Nonetheless, a Scaptomyza pallida P element which differs from the D. melanogaster P element at 20% of its nucleotide positions, is capable of germ-line transformation and can catalyze transposition of both itself and a nonautonomous D. melanogaster P element when transferred to a D. melanogaster M strain (Simonelig and Anxolabéhére 1991). Given the structural similarities between P and hobo, it seems unlikely that the low level of divergence among hobo sequences is due to enormous functional constraints on the molecular evolution of the hobo element.

A more attractive explanation for the similarity among these sequences is that the complete hobo element has spread among the three species in the recent past via some form of horizontal transfer. A similar argument has recently been made to explain the fact that P-element sequences from D. melanogaster and D. willistoni differ at only 1 of 2,907 nucleotide positions, although D. melanogaster and D. willistoni are separated by ≥20 Myr (Daniels et al. 1990b). Both Boussy and Daniels (1991) and Pascual and Periquet (1991) have argued for horizontal transfer of complete hobo elements, on the basis of restriction surveys of D. melanogaster and D. simulans. Their surveys demonstrate that the 2.6-kb XhoI fragment was absent from the oldest available strains and that its gradual appearance over time culminated in its universal presence in modern collections of both species. The limited divergence among hobo sequences can therefore be explained either (a) by the recent entry of the sequences into their respective hosts from a common outside source or (b) by direct transfer from one species to another via either some vector or interspecific hybridization. Three of the species in the D. melanogaster complex—D. simulans, D. mauritiana, and D. sechellia—can form fertile F1 hybrids, and, although D. simulans and D. melanogaster normally form only sterile hybrids, it is probable that at some time in history they were more interfertile than they now are.

One problem with correlating the DNA sequence evidence for the horizontal transfer of hobo with the evidence from restriction surveys is that of time scale. Restriction-survey data suggest that the complete hobo element was not present in D. melanogaster before ~50 years ago (Boussy and Daniels 1991; Pascual and Periquet 1991). The exact date of appearance of hobo is uncertain for D. simulans (Boussy and Daniels 1991) but cannot be much earlier, unless polymorphism for the presence of hobo is able to persist for extended periods of time. However, if rates of DNA sequence divergence of hobo elements are at all similar to those of ordinary genes, the time of the most recent common ancestor of these hobo sequences could be as early as 100,000 years ago or more, based on an estimated rate of silent substitution of
The data at hand do not, however, support any unambiguous interpretation about the time or direction of horizontal transfer events among these species. Before such interpretation can be made, sequences of more hobo elements from each species will have to be made available, to delineate levels of intra- versus interspecific variation. Data on intraspecific sequence variation will also be necessary to rule out the formal possibility that lineage sorting of ancestral polymorphism, rather than horizontal transfer, is responsible for the lack of resolution of the hobo “gene tree” (Pamilo and Nei 1988). However, lineage sorting is typically a problem only when levels of intraspecific polymorphism are high, which does not seem to be the case for hobo.

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